Biomarkers for Individual Susceptibility to Carcinogenic Agents: Excretion and Carcinogenic Risk of Benzo[*a*]pyrene Metabolites

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In rats exposed to a single intraperitoneal dose of 200 mg/kg of the environmental carcinogen benzo[*a*]-pyrene (BP) in sunflower oil, significant individual variations in excretion of the BP activation (BP-7,8-diol) and deactivation (3-OH-BP) derivatives were found. Most rats developed peritoneal sarcomas. Only the levels of BP-7,8-diol excreted in the urine correlated directly with the latency of tumor formation. After a similar exposure to a dose of 100 mg/kg BP, *Macaca fascicularis* monkeys excreted smaller quantities than rats of both metabolites. After rats were given 10 intraperitoneal injections each of 10 mg/kg of BP in a water-lipid emulsion, the excreted levels of both metabolites after the first, fifth, and tenth injection were lower than those of the rats that received 200 mg/kg. BP metabolites were also detected in the urine of lung cancer patients who were heavy smokers. The applicability of monitoring the excretion of the BP metabolites to predicting individual cancer risk is discussed.

Introduction

The susceptibility markers that we are currently developing for predicting individual human response to carcinogenic exposures include the following end points: *a*) endogenous formation of carcinogens (*1*), *b*) excretion of metabolites of carcinogens (*2*), and *c*) formation of adducts of carcinogens with various cellular targets, predominantly DNA, and efficiency of DNA repair (*3,4*).

In this communication, we present recent data that may contribute to the development of markers of individual susceptibility to a powerful environmental carcinogen, benzo[*a*]pyrene (BP). In rats, we have monitored excretion of its activation (BP-7,8-diol) and deactivation (3-OH-BP) derivatives and studied the correlation between individual patterns of metabolite excretion and the carcinogenic effect of BP. The pattern of excretion of BP metabolites in rats was also compared with that in monkeys, as they are most relevant to the human situation.

Materials and Methods

Experiments were carried out on male outbred LIO rats (*5*), weighing approximately 150 g, and male *Macaca fascicularis* monkeys weighing 4–6 kg. Group 1 (19 rats) received a single intraperitoneal injection of BP in sunflower oil at a dose of 200 mg/kg; group 2 (4 rats) and five monkeys received a dose of 100 mg/kg. After exposure, the animals were placed in individual metabolic cages, and feces and urine were collected daily for 5–30 days (see Table 1) on ice and stored at −4°C. BP-7,8-diol and 3-OH-BP were extracted from urine and feces according to a modification of the technique of Camus et al. (*6*) on an Amberlite XAD-2 column, deconjugated and quantified spectrofluorimetrically (*7*). Rats in group 1 were maintained to monitor the development of tumors. Group 3, consisting of 10 rats which are currently under observation for tumor development, were given 10 intraperitoneal injections of a water–lipid emulsion of BP at a dose of 10 mg/kg, with 10-day intervals between injections, and BP metabolites were quantified in the excreta after the first, fifth, and tenth injections.

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Individual patterns of BP metabolite excretion and tumor latency in rats in group 1 were correlated using the parametric approach of regression analysis. The relationship between quantities of excreted BP metabolites and tumor latency was also evaluated by a modification of Kaplan–Meier analysis (*8*).

Results

Individual and Species-Specific Peculiarities of BP Metabolite Excretion

Peak concentration of both metabolites were observed in rats of group 1 (Table 1) 2–3 days after exposure, followed by a slow decrease: by 30 days after exposure, neither metabolite was found in the excreta. Both BP metabolites were excreted predominantly with the feces. Individual variations in total excretion of BP metabolites over 5, 8, and 15 days were considerably smaller than daily variations.

Strong correlations were observed only between fecal excretion of BP-7,8-diol and 3-OH-BP and between excretion of 3-OH-BP in urine and feces (correlation coefficients ranged from 0.67 to 0.91). The mechanisms of this phenomenon and its possible biological significance are unclear.

After multiple exposures to a lower dose of BP (group 3), the levels of excreted BP metabolites were lower than those of rats in group 1, decreasing progressively with progressive doses. This result may be due to an age-associated decrease in the activity of drug-metabolizing enzymes (*9*) and/or exhaustion of these enzymes by the preceding exposures.

Monkeys were weaker metabolizers of BP than rats: in monkeys, the total excretion of BP-7,8-diol in feces over 5 days ranged from 14.6 to 48.5 μg/kg body weight (mean = 29.4), and that of 3-OH-BP from 0.8 to 1.8 (mean = 1.2), whereas in the rats the corresponding values were 79.9–163.6 (*mean* = 17.5) and 14.0–25.2 (*mean* = 21.8). The ratio of BP-7,8-diol to 3-OH-BP in the feces of monkeys, however, was as much as 5–6 times higher than that in the rats.

Comparisons of Individual Patterns of BP Metabolite Excretion in Rats Exposed to a Single Dose of BP and Its Subsequent Carcinogenic Effect

In rats of group 1, the first tumor was observed on day 123 after exposure in one of 16 rats that survived this long. Ten rats developed peritoneal tumors with local dissemination, all of which appeared to be malignant fibrous histiocytomas. The individual patterns of total excretion of BP metabolites over 5 days after exposure and tumor latency (time of death) are presented in Table 2. Maximal correlations between BP metabolite excretion and tumor latency were detected with an exponential model for total excretion of BP-7,8-diol over 5 days in urine (Table 3). Evaluation of the probability of tumor appearance during the period of observation on the basis of the quantities of excreted metabolites if highly approximative because of

Table 1. Excretion of benzo[*a*]pyrene (BP) metabolites (μg/kg body weight) by rats exposed intraperitoneally to BP.

		Urine				Feces			
	Days after	BP-7,8-diol		3-OH-BP		BP-7,8-diol		3-OH-BP	
Group	exposure	Mean ± SEM	Range	Mean ± SEM	Range	Mean ± SEM	Range	Mean ± SEM	Range
1	1	8.7 ± 1.4	1.5–24.0	3.9 ± 0.6	1.5–10.4	19.8 ± 2.6	0–41.1	6.9 ± 1.0	2.2–18.9
	2	15.0 ± 1.9	1.9–32.4	5.4 ± 0.7	1.5–13.1	56.5 ± 7.5	19.4–138.9	9.9 ± 1.5	2.6–26.7
	3	14.6 ± 1.8	4.1–32.8	6.2 ± 1.6	1.4–27.8	40.7 ± 1.7	27.5–54.3	8.9 ± 0.8	3.2–16.1
	4	11.3 ± 1.2	2.3–20.7	5.0 ± 1.1	1.9–19.5	43.7 ± 5.1	14.6–96.1	9.6 ± 1.0	2.1–18.6
	5	10.8 ± 1.5	0.7–25.1	4.2 ± 0.6	1.4–11.2	27.5 ± 6.0	2.8–98.8	7.7 ± 0.9	1.6–15.8
	Total over 5 days	59.8 ± 5.0	16.0–96.7	28.9 ± 3.0	8.0–56.1	188.2 ± 17.1	74.5–348.4	43.0 ± 5.0	13.8–93.4
	6	5.3 ± 3.1	1.8–11.0	2.6 ± 0.2	2.5–3.0	30.4 ± 8.3	20.7–44.8	9.1 ± 3.2	5.7–15.0
	7	4.9 ± 2.2	1.5–7.8	2.6 ± 0.3	2.1–2.9	30.0 ± 12.5	10.1–46.7	8.4 ± 1.9	5.6–11.3
	8	*7.1 ± 2.5*	*3.3–10.6*	*2.5 ± 0.3*	*2.2–2.9*	*11.6 ± 2.5*	*7.3–14.4*	*4.0 ± 0.2*	*3.6–4.3*
	Total over 8 days	77.1 ± 27.4	45.6–125.7	36.6 ± 3.4	18.6–28.4	260.2 ± 8.7	211.4–236.7	64.5 ± 6.5	50.5–69.6
	9	5.6 ± 3.1	0.3–9.2	2.8 ± 0.5	2.1–3.1	6.7 ± 2.4	3.6–10.6	3.3 ± 0.7	2.5–4.5
	10	8.4 ± 1.2	6.3–9.7	2.7 ± 0.2	2.4–2.9	7.7 ± 1.0	6.7–9.6	3.2 ± 0.6	2.4–4.0
	11	4.6 ± 2.6	1.1–8.7	2.5 ± 0.2	2.1–2.8	9.1 ± 1.7	7.1–12.0	3.1 ± 0.2	2.7–3.3
	12	4.3 ± 0.6	3.6–5.2	2.6 ± 0.2	2.2–2.8	1.5 ± 0.2	1.3–1.8	2.5 ± 0.2	2.1–2.8
	13	3.5 ± 2.2	0.9–7.3	2.5 ± 0.1	2.3–2.7	5.3 ± 1.6	2.7–7.4	2.5 ± 0.1	2.3–2.7
	14	2.8 ± 0.7	2.2–4.1	2.2 ± 0.5	1.3–2.6	3.2 ± 2.3	0.4–7.1	2.3 ± 0.1	2.2–2.5
	15	4.8 ± 0.6	4.1–5.9	2.5 ± 0.2	2.2–2.7	1.7 ± 1.0	0–2.9	2.3 ± 0.2	1.9–2.5
	Total over 15 days	111.1 ± 36.4	45.6–151.2	50.3 ± 5.1	33.2–48.1	295.4 ± 9.7	245.7–273.8	79.9 ± 8.2	66.7–90.6
3[a]	a	15.9 ± 3.1	4.7–32.0	2.1 ± 0.2	1.3–4.3	43.2 ± 0.5	12.7–134.2	8.3 ± 0.4	7.2–11.5
	b	4.3 ± 0.5	1.8–7.1	1.1 ± 0.1	0.7–1.5	27.0 ± 0.7	7.7–99.0	7.8 ± 0.7	3.4–10.8
	c	5.0 ± 1.3	0.2–15.2	0.8 ± 0.1	0.5–1.3	25.8 ± 0.1	11.7–51.2	3.5 ± 0.3	2.5–5.2

[a]a, 8 days after first injection; b, 8 days after fifth injection; c, 8 days after tenth injection.

Table 2. Total excretion of benzo[*a*]pyrene (BP) metabolites (μg/kg body weight) over 5 days after intraperitoneal injection of BP (200 mg/kg) and tumor latency in rats.

Urine		Feces		Tumor latency, days
BP-7,8-diol	3-OH-BP	BP-7,8-diol	3-OH-BP	
63.6	8.1	159.3	26.8	123
68.7	56.1	358.4	85.6	151
75.2	9.0	164.1	17.3	153
30.2	10.1	64.5	13.9	153
63.6	54.9	289.7	75.8	166
58.1	13.2	143.5	16.8	196
60.9	38.2	190.6	56.1	211
69.2	24.0	198.4	53.5	212
96.7	21.8	124.2	20.9	239
74.0	15.3	133.9	22.8	388

the limited number of observations; however, the probabilities decreased proportionally with increases in total excretion of BP-7,8-diol (Table 4). Thus, both statistical approaches suggest that only the levels of BP-7,8-diol excreted with urine are correlated directly with the latency of tumor appearance.

Conclusions and Future Perspectives

These comparative metabolic studies show significant individual and species-specific variation in the excretion of carcinogenic activation and deactivation products of BP. It is still unclear, however, whether these parameters of BP metabolism can be indicative of a high individual carcinogenic risk. Camus et al. (*6*) demonstrated that mice of a strain responsive to the carcinogenic effect of BP produced larger quantities of total BP oxidized metabolites and had a lower ratio of BP diols to oxidized metabolites relative to mice of a nonresponsive strain. Our experiment in rats demonstrates that the indicative marker of individual susceptibility to BP carcinogenesis is urinary excretion of BP-7,8-diol. This observation requires confirmation, which is the aim of our experiment with multiple exposures to BP. Comparisons with other species, including primates, will allow better extrapolation of animal data to the human situation.

The applicability of this approach for monitoring BP metabolite excretion in humans is confirmed by our ongoing study, in which BP-7,8-diol and 3-OH-BP were detected in the urine of lung cancer patients who were heavy smokers at concentrations comparable with those in the rats in the study described above. Assuming that DNA adduct formation and repair are the most reliable predictors of carcinogenic risk (*10*), we are comparing their levels in liver of rats exposed to BP with levels of BP metabolites in the urine of the same animals.

Individual markers of susceptibility to various carcinogens could be recognized at any stage of carcinogenesis. Most markers are associated with the initiation stage; it will be useful to identify possible markers of the promotion stage of carcinogenesis.

Table 4. Probability of tumor development in rats with different levels of excretion of benzo[*a*]pyrene-7,8-diol in urine.

Days after exposure	Level of excretion (μg/kg body weight)		
	<60	60–70	>70
0–130	0.33	0.00	0.00
0–160	0.67	0.25	0.33
0–190	0.67	0.50	0.33
0–210	1.00	0.50	0.33
0–240	1.00	1.00	0.67
0–390	1.00	1.00	1.00

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REFERENCES

1. Dikun, P. P., Ermilov, V. B., and Shendrikova, I. A. Some approaches to prevention of endogenous formation of *N*-nitrosamines in humans. In: Relevance to Human Cancer of *N*-Nitroso Compounds, Tobacco Smoke and Mycotoxins (I. K. O'Neill, J. Chen, and H. Bartsch, Eds.), IARC Scientific Publication No. 105, International Agency for Research on Cancer, Lyon, 1991, pp. 552–557.
2. Likhachev, A. J., Beniashvili, D. Sh., Dikun, P. P., Savochkina, I. V., and Zabezhinski, M. A. Experimental approach to predicting individual susceptibility to carcinogens: non-invasive test for benzo[a]pyrene metabolism. Cancer Res. Clin. Oncol. 116 (suppl.): 79 (1990).
3. Wild, C. P., Degan, P., Bresil, H., Serres, M., Montesano, R., Likhavchev, A., and Gershanovitch, M. Quantitation of 7-methyldeoxyguanosine in peripheral blood cell DNA after exposure to methylating agents. Proc. Am. Assoc. Cancer Res. 28: 260 (1988).

Table 3. Coefficients of correlation between individual patterns of excretion of benzo[*a*]pyrene (BP) metabolites at different intervals after exposure and tumor latency.

Time after exposure, days	Linear model $Y = a + bx$				Exponential model $Y = exp\ (a + bx)$			
	Urine		Feces		Urine		Feces	
	BP-7,8-diol	3-OH-BP	BP-7,8-diol	3-OH-BP	BP-7,8-diol	3-OH-BP	BP-7,8-diol	3-OH-BP
1	−0.02	−0.05	+0.03	−0.23	+0.04	+0.02	+0.07	−0.21
2	+0.45	+0.05	−0.07	−0.21	+0.54	+0.13	−0.05	−0.21
3	−0.02	−0.19	+0.20	−0.05	−0.01	−0.17	+0.21	+0.05
4	+0.19	−0.11	−0.42	−0.27	+0.09	−0.08	−0.43	−0.23
5	+0.37	−0.13	−0.42	−0.18	+0.44	−0.05	−0.42	−0.12
1+2	+0.40	+0.22	−0.06	−0.22	+0.50	+0.10	−0.03	−0.21
1+2+3	+0.36	−0.12	−0.01	−0.17	+0.46	−0.06	+0.02	−0.15
1+2+3+4	+0.40	−0.12	−0.17	−0.20	+0.43	−0.07	−0.15	−0.18
1+2+3+4+5	+0.42	−0.12	−0.26	−0.21	+0.47	−0.07	−0.25	−0.17

4. Likhachev, A., Zhukovskaya, N., Anisimov, V., Hall, J., and Napalkov, N. Activity of 06-alkylguanine-DNA alkyltransferase in the liver, kidney and white blood cells of rats of different ages. In: Relevance to Human Cancer of *N*-Nitroso Compounds. Tobacco Smoke and Mycotoxins (I. K. O'Neill, J. Chen, and H. Bartsch, Eds.), IARC Scientific Publication No. 105, International Agency for Research on Cancer, Lyon, 1991, pp. 407–411.
5. Anisimov, V. N., Pliss, G. B., Iogannsen, M. G., Popovich, I. G., and Romanov, K. P. Spontaneous tumors in outbred LIO rats. J. Exp. Clin. Cancer Res. 8: 254–262 (1989).
6. Camus, A. M., Aitio, A., Sabadie, N., Wahrendorf, J., and Bartsch, H. Metabolism and urinary excretion of mutagenic metabolites of benzo[a]-pyrene in C57 and DBA mice strains. Carcinogenesis 5: 33–39 (1984).
7. Savochkina, I. V., Dikun, P. P., Zabezhinski, M. A., Yamshanov, V. A., and Likhachev, A. J. Individual peculiarities of excretion of benzo[a]pyrene metabolites in rats. Vopr. Oncol. 36: 1221–1226 (1990).
8. Kaplan, E. L., and Meier, P. Nonparametric estimation from incomplete observations. J. Am. Stat. Assoc. 53: 457–481 (1958).
9. Anisimov, V. N. Carcinogenesis and Aging, Vol. 2. CRC Press, Boca Raton, FL, 1987.
10. Bartsch, H., Hemminki, K., and O'Neill, I. K., Eds. Methods for Detecting DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention. IARC Scientific Publication No. 89, International Agency for Research on Cancer, Lyon, 1988.